Jun 26, 2019 Version 3

Reverse transcription using SuperScript IV V.3

DOI
dx.doi.org/10.17504/protocols.io.4sugwew

Amin Mahpour[1]

[1]Harvard Medical School

Amin Mahpour
Harvard Medical School, Massachusetts General Hospital

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.4sugwew

External link: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205608

Protocol Citation: Amin Mahpour 2019. Reverse transcription using SuperScript IV. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4sugwew

Manuscript citation:
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0205608

Protocol status: Working
We use this protocol and it's working

Created: June 26, 2019

Last Modified: June 26, 2019

Protocol Integer ID: 25140

Keywords: Using SuperScript IV to produce cDNA from RNA

Guidelines

Use high quality RNA as the substrate.
Keep RNA on ice at all times.
Use RNase free water.

Materials

MATERIALS

SuperScript™ IV Reverse Transcriptase **Thermo Fisher Scientific** **Catalog #**18090050

1 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.

	Reagent	Amount (uL)
	Primer (Random or dT)	0.5
	dNTP (10mM)	1
	RNA	11

2 Incubate the mixture at 72 °C for 00:02:00. Then, incubate samples on ice for few minutes.

Note

This step allows denaturation of RNA and proper priming for the downstream cDNA synthesis.

3 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.

	Reagent	**Amount (uL)**
	RT Buffer (5x)	4
	DTT(10mM)	1
	RNase Inhibitor	1

Add the 6uL to the 12.5uL mix from Step 3.

4 Incubate the samples at 37 °C for 00:05:00. Then, add 1.5uL SuperScript RT IV enzyme to the reaction and mix well.

5 Incubate the samples using the following incubation settings:

	Temp (C)	Time (minutes)
	25	5
	45	40
	55	10
	75	10

5.1 Add 1uL RNase H to the cDNA samples and incubate at 37 °C for 00:20:00 .

5.2 Dilute the cDNA samples using Nuclease free water.